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APPLICATION

FOR

UNITED STATES LETTERS PATENT

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TITLE : AGE-1 POLYPEPTIDES AND RELATED MOLECULES AND METHODS

AGE-1 POLYPEPTIDES AND RELATED MOLECULES AND METHODS

Cross Reference to Related Applications

This application claims benefit from provisional application 60/023,382 filed on August 7, 1996.

Background of the Invention

This invention relates to polypeptides and nucleic acid sequences involved in aging, as well as methods for their use.

As the average age of the population of the United States and other countries increases, there is a growing interest in efforts to delay the aging process. It has long been accepted that environmental factors which trigger DNA damage or are otherwise toxic to cells might negatively influence longevity. Increasingly, the role of genetics in this process has become accepted as well, and has resulted in a search for genes which participate in and control aging or senescence. These genes are valuable because they may encode therapeutic products which retard senescence or death. Alternatively, these proteins may be used as targets for the design or isolation of antagonist-type drugs which themselves prolong life-span or delay the onset of age-related conditions.

Summary of the Invention

In general, the invention features a substantially pure preparation of AGE-1 polypeptide, the polypeptide having at least 50% (and preferably 70% or 90%) amino acid sequence identity to the polypeptide of Figure 6 (SEQ ID NO: 1). Preferably, the AGE-1 polypeptide includes identical amino acids in equivalent positions to 50% (and preferably 70% or 90%) of the following amino acids of Figure 6 (SEQ ID NO: 1): amino acids Gly-32, Leu-73, His-78, Phe-81, Glu-109, Phe-114, Leu-123, Leu-125, Phe-129, Lys-181, Ser-208, Lys-211, Arg-321, Leu-325, Leu-351, Ser-355, Met-373, Leu-381, Leu-393, Thr-432, Tyr-451, Glu-475, Pro-507, Ile-514, Gly-518, Glu-530, Val-538,

Leu-582, Tyr-606, Pro-643, Phe-665, Leu-744, Leu-745, Arg-762, Leu-789, Arg-794, Ala-827, Arg-829, Trp-835, Ser-842, Asn-905, Gly-917, Asp-975, Ile-990, Asp-1006, His-1020, Lys-1104, Thr-1105, Gly-1130, Phe-1140, and Lys-1144. An alanine at equivalent amino acid 827 is particularly preferred. In other preferred embodiments, the AGE-1 polypeptide is derived from an animal, for example, *Caenhorabditis elegans* or a mammal, for example, a human.

The invention also features useful fragments of AGE-1 polypeptides; in particular, preferred fragments include amino acids 387-641, 387-1146, 1-130, 1-150, 1-658, or 1-404 of Figure 6 (SEQ ID NO: 1).

In related aspects, the invention features purified DNA (for example, cDNA) which encodes any of the AGE-1 polypeptides described above or which includes an AGE-1 nucleic acid sequence which is at least 30% (and preferably 40%, 50%, 70%, 80%, or 90%) identical to the nucleic acid sequence of Figure 4 (SEQ ID NO: 2). In addition, the invention features a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides 64 to 852 Figure 4 (SEQ ID NO: 2); a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides ~~nucleotides~~ 865 to 912 of Figure 4 (SEQ ID NO: 2); a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides 919 to 975 of Figure 4 (SEQ ID NO: 2); a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides 1003 to 3090 of Figure 4 (SEQ ID NO: 2); a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides 3094 to 3501 of Figure 4 (SEQ ID NO: 2); and a purified DNA including an AGE-1 nucleic acid sequence substantially identical to nucleotides 2620 to 2655 of Figure 4 (SEQ ID NO: 2). The invention also features a vector and a cell, each of which includes a purified AGE-1 DNA of the invention; and a method of producing a recombinant AGE-1 polypeptide involving providing a cell transformed with DNA encoding an AGE-1 polypeptide positioned for expression in the cell, culturing the

transformed cell under conditions for expressing the DNA, and isolating the recombinant AGE-1 polypeptide. The invention further features recombinant AGE-1 polypeptide produced by such expression of a purified DNA of the invention, and a substantially pure antibody that specifically recognizes and binds an AGE-1 polypeptide.

In addition, the invention features methods of identifying AGE-1 modulatory compounds. The first method involves the identification of a modulatory compound that is capable of decreasing the expression of an AGE-1 gene, involving (a) providing a cell expressing the AGE-1 gene and (b) contacting the cell with a candidate compound, a decrease in AGE-1 expression following contact with the candidate compound identifying a modulatory compound. The second method also involves the identification of a modulatory compound which is capable of decreasing AGE-1 activity (for example, kinase activity); this method involves (a) providing a cell expressing an AGE-1 polypeptide and (b) contacting the cell with a candidate compound, a decrease in AGE-1 activity (for example, kinase activity) following contact with the candidate compound identifying a modulatory compound.

In preferred embodiments of both methods, the AGE-1 gene encodes or AGE-1 polypeptide includes an amino acid sequence that is at least 50% (and preferably 70% or 90%) identical to the amino acid sequence shown in Fig. 6 (SEQ ID NO: 1); and the AGE-1 gene or AGE-1 polypeptide is from an animal (for example, *C. elegans*), and preferably a mammal (for example, a human). In other preferred embodiments, the method is carried out in a nematode or other animal, or the method involves assaying AGE-1 activity in vitro.

The invention further features modulatory compounds identified by the above methods, as well as a method for increasing longevity in a mammal that involves administering such a compound to a mammal (for example, a human).

In addition, the invention features a method of determining the longevity of an animal. The method involves measuring AGE-1 gene expression or AGE-1 activity (for

example, kinase activity) in a sample from the animal, with a decrease in AGE-1 expression or activity relative to a wild-type sample being an indication that the animal has increased longevity.

In preferred embodiments, the animal is a mammal (for example, a human); AGE-1 gene expression is measured by assaying the amount of AGE-1 polypeptide in the sample (for example, by immunological methods); or AGE-1 gene expression is measured by assaying the amount of AGE-1 mRNA in the sample (for example, by hybridization or PCR techniques using an AGE-1-specific nucleic acid sequence).

Kits for carrying out the above methods are also included in the invention. Such kits preferably include a substantially pure antibody that specifically recognizes and binds an AGE-1 polypeptide and may also include means for detecting and quantitating antibody binding. Alternatively, the kit may include all or a fragment of an AGE-1 nucleic acid sequence useful for hybridization or PCR purposes and may also include means for detecting and quantitating the products of the hybridization or amplification.

By "AGE-1 polypeptide" is meant a phosphatidylinositol 3-kinase (PI 3-kinase) involved in the control of senescence.

By "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

By "substantially pure" is meant a preparation which is at least 60% by weight (dry weight) the compound of interest, e.g., the AGE-1 polypeptide or AGE-1-specific antibody. Preferably the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "purified DNA" is meant DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is

derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By a "substantially identical" nucleic acid is meant a nucleic acid sequence which encodes a polypeptide differing only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the function of the polypeptide (assayed, e.g., as described herein). Preferably, the encoded sequence is at least 30% identical at the amino acid level to the sequence of Fig. 6 (SEQ ID NO: 1) or an AGE-1 domain thereof (as described herein). In other preferred embodiments, the encoded sequence is at least 40%, preferably 50%, more preferably 60%, and most preferably 70% identical at the amino acid level to the sequence of Fig. 6 (SEQ ID NO: 1) or an AGE-1 domain thereof. In still other preferred embodiments, the encoded sequence is at least 80%, preferably 85%, more preferably 90%, and most preferably 95% identical at the amino acid level to the sequence of Fig. 6 (SEQ ID NO: 1) or an AGE-1 domain thereof. If nucleic acid sequences are compared a "substantially identical" nucleic acid sequence is one which is at least 30%, more preferably 40%, and most preferably 50% identical to the sequence of Fig. 4 (SEQ ID NO: 2) or a sequence encoding an AGE-1 domain thereof. In other preferred embodiments, the substantially identical nucleic acid sequence is one which is at least 75%, more preferably 85%, and most preferably 95% identical to the sequence of Fig. 4 (SEQ ID NO: 2) or a sequence encoding an AGE-1 domain. The length of nucleic acid sequence comparison will generally be at least 50 nucleotides, preferably at least 60

nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Homology is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705, BLAST, or PILEUP/PRETTYBOX programs). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of AGE-1 protein).

By "purified antibody" is meant antibody which is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, antibody.

By "specifically binds" is meant an antibody which recognizes and binds an AGE-1 polypeptide but which does not substantially recognize and bind other molecules in a sample (e.g., a biological sample) which naturally includes AGE-1 polypeptide. An antibody which "specifically binds" AGE-1 is sufficient to detect an AGE-1 protein product in such a biological sample using one or more of the standard immunological techniques available to those in the art (for example, Western blotting or immunoprecipitation).

By "longevity" is meant rate of senescence and/or life-span.

By "relative to a wild-type sample" is meant relative to an equivalent tissue sample from one or more individuals of average life-span. An individual of average life-

span may be determined by an analysis of AGE-1 levels in a statistically significant number of members of a population.

By "immunological methods" is meant any assay involving antibody-based detection techniques including, without limitation, Western blotting, immunoprecipitation, and direct and competitive ELISA and RIA techniques.

By "means for detecting" is meant any one or a series of components that sufficiently indicate a detection event of interest. Such means involve at least one label that may be assayed or observed, including, without limitation, radioactive, fluorescent, and chemiluminescent labels.

By "AGE-1 RNA" is meant messenger RNA transcribed from an AGE-1 DNA sequence.

By "hybridization techniques" is meant any detection assay involving specific interactions (based on complementarity) between nucleic acid strands, including DNA-DNA, RNA-RNA, and DNA-RNA interactions. Such hybridization techniques may, if desired, include a PCR amplification step.

By "kinase activity" is meant AGE-1-mediated production of phosphatidylinositol P_3 (PIP_3).

By a "modulatory compound", as used herein, is meant any compound capable of either decreasing AGE-1 expression (i.e., at the level of transcription, translation, or post-translation) or decreasing AGE-1 protein activity (i.e., the amount of activity, for example, kinase activity, per unit amount of AGE-1 protein).

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

Brief Description of the Drawings

Figure 1 is a graph showing defective maternal *age-1* gene activity in *age-1(hx546)* animals. The *age-1(mg44)* chromosome in the figure is marked in *cis* with *sqt-*

1(sc13). age-1(mg44) progeny of *age-1(mg44)* homozygous parents are shown to the left. Progeny of *age-1(hx546)/sqt-1(sc13) age-1(mg44)* heterozygous parents are shown in the middle. Progeny of *+/sqt-1(sc13) age-1(mg44)* heterozygous parents are shown to the right. The *sqt-1(sc13)* genetic marker was used to identify homozygous *age-1(mg44)* progeny. Similar results were observed when another marker, *unc-4*, was used to mark another *age-1* allele, *m333*, that is also likely to be a null allele, and *mg109* (data not shown). Animals were grown at 25°C in the experiment shown. Note that *sqt-1(sc13) age-1(mg44)* progeny of *age-1(hx546)/sqt-1(sc13) age-1(mg44)* arrest development as dauer larvae with 100% penetrance at 25°C. This is the same as control *age-1(mg44)* daughters of *age-1(mg44)* parents. In the case of *age-1(mg44)* daughters of *age-1(mg44)* parents, we also noted 6% (n=30) of these animals arrest at the L1 or L2 stage. Many of these arrested animals eventually grow to dauer-like larvae and sterile adults. In contrast, 1% of *sqt-1(sc13) age-1(mg44)* progeny of *+/sqt-1(sc13) age-1(mg44)* arrest development as dauer larvae at 25°C. *age-1(hx546)/sqt-1(sc13) age-1(mg44)* progeny form fertile adults, just like *+/ sqt-1(sc13) age-1(mg44)* progeny. This shows that *age-1(hx546)* expresses sufficient zygotic but not maternal levels of *age-1* for non-dauer growth.

At 20°C, fewer of the *sqt-1(sc13)age-1(mg44)* progeny of *age-1(hx546)/sqt-1(sc13) age-1(mg44)* arrest development as dauer larvae; most continue development as dauer-like animals that are dark and sterile. Such temperature dependent dauer arrest has previously been noted for *age-1(mg44)* (Gottlieb, S. & Ruvkun, G. *Genetics* **137,** 107-120 (1994)). We cannot attribute the more severe phenotype at high temperatures to any temperature sensitivity of *age-1(hx546)* because even wild type dauer formation is a temperature dependent process (Golden, J.W. & Riddle, D.L., *Proc. Natl. Acad. Sci.* **81**:819-823 (1984)). At 20 degrees, unlike 25 degrees, there is a detectable maternal *age-1* activity in *age-1(hx546)*: 4% of *sqt-1(sc13) age-1(mg44)* progeny of *age-1(hx546)/sqt-1(sc13) age-1(mg44)* parents are fertile (data not shown), unlike *age-1(mg44)* daughters of *age-1(mg44)* mothers (Gottlieb, S. & Ruvkun, G. *Genetics* **137,** 107-120 (1994)). In

contrast, at 20 degrees, 93% of *sqt-1(sc13) age-1(mg44)* progeny of +/ *sqt-1(sc13) age-1(mg44)* parents are fertile.

In this Figure, superscript [a] indicates that one fertile Sqt adult was a recombinant *sqt-1(sc13) age-1(mg44)/sqt-1(sc13) age-1(hx546)*. Two other *sqt-1(sc13) age-1(mg44)/sqt-1(sc13) age-1(hx546)* recombinants were picked from the same parental strains in other experiments (data not shown). Superscript [b] indicates that all fertile Sqt adults produced all Sqt dauers in the F3 except for three *sqt-1(sc13) age-1(mg44)/sqt-1(sc13)* recombinants.

The genotype of the recombinants was determined by picking individual animals from the original non-dauer Sqt and then examining its brood. Three *sqt-1(sc13) age-1(hx546)/sqt-1(sc13) age-1(mg44)* recombinants map *age-1(hx546)* to the right of *sqt-1(sc13)*, because these recombinants show that each time *sqt-1(sc13)* was recombined away from *age-1(mg44)* in the *age-1(hx546)/sqt-1(sc13) age-1(mg44)* heterozygote, *age-1(hx546)* was recovered on the *sqt-1* recombinant chromosome. *age-1(hx546)* was also three-factor mapped relative to *sqt-1(sc13)* and *lin-29*; two Lin non-Sqt recombinants were Age and 10 Sqt non-Lin recombinants were non-Age, based on lack of maternal rescue of *age-1(m333)*. This shows that *age-1* maps to the left of *lin-29*.

To carry out these experiments, *age-1(hx546)* males were mated into *sqt-1(sc13) age-1(mg44)* hermaphrodites at 20°C. F1 Non-Sqt Non-Daf cross progeny (3/3) were picked to separate plates. Individual heterozygotes were singled to plates at either 25, 20, or 15 degrees and transferred daily (20° and 25°) or every 2-3 days (20° and 15°). Worms were then counted and scored for dauer and non-dauer either 3 days later (25°), 4 days later (20°), or 7 days later (15°). For genetic mapping, *age-1(hx546)* males were mated with *sqt-1(sc13) lin-29(n333)/mnC1* hermaphrodites, and individual wild type cross progeny were picked to separate plates. Plates that segregated wild type, Sqt Lin animals, and no progeny bearing mnC1 were examined for recombinants that were Sqt-non-Lin and Lin-non-Sqt. Putative recombinants were then picked to individual

plates, and progeny from the heterozygous recombinant were singled to establish a homozygous recombinant strain. 10 Sqt-non-Lin and 2 Lin-non-Sqt recombinants were then scored for failure to maternally complement *age-1(m333)* for dauer constitutivity at 25°C. *unc-4(e120) age-1(m333)/mnC1* males were mated with the recombinants. The Lin non-Sqt recombinants were opened at the vulva by a microinjection needle prior to mating. Wild-type hermaphrodite cross progeny were singled to 25°C, and their progeny were scored for whether *unc-4(e120) age-1(m333)* homozygous progeny arrested as dauer larvae.

Figure 2A is a diagram showing a physical/genetic map of the *age-1* region, with genetic left oriented towards the left. *age-1* is transcribed from right to left on this genetically oriented map. Cosmid and YAC clones in the *sqt-1 lin-29* interval were placed by the *C. elegans* genome project. *mnDf75*, *mnDf76*, and *mnDf86*, all of which complement *age-1* and fail to complement *sqt-1*, break in cosmid C24F2, as detected by Southern blots to Df/mnC1 DNAs using this cosmid as a probe (data not shown). The breakpoint in *mnDf76*, but not the breakpoints in *mnDf75* or *mnDf86*, were also detected with cosmid W10C3, which partially overlaps C24F2 from the left. Thus *age-1* must be located to the right of the breakpoints of these deficiencies. PCR analysis using *kin-6* primers of embryos homozygous for *mnDf90*, which fails to complement both *sqt-1* and *age-1*, shows that this deficiency deletes DNA to the left of *kin-6*, placing *age-1* to the left of *kin-6*. Southern blots using cosmid B0334 as a probe detect a breakpoint in DNAs isolated from *age-1(mg55)*/mnC1 but not other *age-1* alleles or other strains carrying mnC1. The age-1 transcript is not drawn to scale.

Figure 2B is a photograph of a Southern blot analysis showing hybridization of HindIII-digested genomic DNA isolated from wild type (N2) (lane 1), *age-1(mg55)/mnC1* (lane 2), and three Df/mnC1 control strains (lanes 3, 4, and 5) to a probe made from a 4 kb SalI subclone of phage 5B. This probe detects the region that encodes the C-terminal region of AGE-1. The strain bearing *mg55* bears both a 5.2 kb HindIII

fragment from the wild type *age-1* allele on mnC1 and an altered 3.1 kb HindIII fragment from the *age-1(mg55)* allele, indicating that the *mg55* breakpoint was in or near the rightmost portion of cosmid B0334.

Figure 2C is a diagram showing the gene structure of *age-1* displayed next to the genomic and cDNA clones used to isolate the gene. The gene is flipped 180° relative to the genetic map, so that transcription is oriented left to right. White boxes indicate predicted untranslated regions. Periods indicate regions where sequence was obtained by RTPCR to verify splice junctions and to obtain the sequence joining cDNAs B and C to A. Independent cDNAs B and C occur in opposite orientations in the lambda vector and thus represent independent cloning events. They end within 30 bp of each other at their N termini, suggesting that they define the bona fide end of the age-1 mRNA. A third cDNA (not shown) ends within 5 bp of cDNA C, and is in the same orientation as cDNA C.

All the deficiencies indicated in Figures 2A, 2B, and 2C were isolated based on their failure to complement *sqt-1*. PCR using primer pairs from both *sqt-1* and *kin-6* was performed on single arrested L1 larvae (*mnDf75, mnDf76, mnDf86*) or from single dead eggs (*mnDf90*) laid by heterozygous deficiency/mnC1 animals. To carry out the single worm/egg PCR protocol, single worms or eggs were picked into 2.5 µl of worm lysis buffer with proteinase K (50mM KCl, 10mM Tris pH 8.2, 2.5mM Mg, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin, 60 µg/ml Proteinase K). Mineral oil was added, and tubes were frozen on dry ice, incubated at 60°C for 1 hour, and at 95°C for 15 minutes to lyse. 2.5 µl of worm lysis buffer was then added, and the reaction was divided into two PCR reactions for *sqt-1* and *kin-6* primers. The *sqt-1* primers were: CTCTGGTTCATTTCCCAACC (SEQ ID NO: 3) and TGTAACTCACCTAGTCTTCG (SEQ ID NO: 4). The *kin-6* primers were: AACAATTACAGGCCGATCC (SEQ ID NO: 5) and ATGCCACGCAAGAAACTCAC (SEQ ID NO: 6).

Phages B and C were isolated from the Barstaead random primer cDNA library (RBII) in lambda ACT. Phage A was isolated from the Ahringer staged embryonic library in lambda gt10. Phage Ø5B was isolated from the Browning genomic library in the lambda Dash vector. RNA for the RTPCR experiments was prepared using guanidinium thiocyanate from mixed stage worms. cDNA preparation and anchor ligation were performed using the 5'-Amplifinder Race kit from Clontech. 36 μg of total RNA was reverse transcribed using a primer specific to *age-1* (sequence: GAAAAGATGGAATGTGACCG) (SEQ ID NO: 7). PCR was performed with the anchored primer from the kit and with a primer of sequence: ATCTGAAGCGTTCTTATATC (SEQ ID NO: 8) (which we later found to have an error). Nested PCR was performed using the anchored PCR primer again and internal primer: TGCTCCATTTTCTCCGATCC (SEQ ID NO: 9).

Figure 3 is the amino acid sequence of *age-1* (SEQ ID NO: 1), as determined by sequencing cDNA clones, amplified reverse transcribed PCR fragments, and genomic regions described in Figure 1. PCR generated templates were sequenced without subcloning and verified by sequencing multiple isolates. *age-1* alleles were sequenced by PCR amplifying genomic regions and direct sequencing of templates without subcloning. Each mutation detected was verified by sequencing an independently amplified template. The AGE-1 sequence comparisons are to mouse p110α (accession number P42337) (SEQ ID NO: 10), human p110β (accession number P42338) (SEQ ID NO: 11), and human p110γ (accession number P48736) (SEQ ID NO: 12). Regions highlighted in gray and black were picked out by Prettybox, aligned by the Pileup program as having extended regions of high amino acid similarity (Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711). Invariant amino acids in 3 of the 4 sequences are shown as black boxes. Similar amino acids in regions highlighted by 3 of 4 exact matches are highlighted in gray boxes. Allele lesions are shown above the amino acid which was

altered. The asterisks (*) indicate a change to a stop codon. The brackets around *age-1(mg55)* show the region to which we mapped the *age-1(mg55)* breakpoint.

In these experiments, sequencing was carried out with the Promega fmol sequencing kit on cDNAs, RTPCR products, and PCR amplified genomic regions from the *age-1* mutant alleles. The GCG programs, Translate, Pileup, and Prettybox, were then used to analyze the sequence.

Figure 4 is the nucleic acid sequence of an *age-1* cDNA (SEQ ID NO: 2).

Figure 5 is a diagram illustrating an AGE-1 lineup with mouse p110α. The diagram is to scale and extends over the 1146 amino acid AGE-1 sequence. Percentage identities shared between each domain of mouse p110α and the region of AGE-1 aligned with it by the Gap program are given. The domains labelled ras and p85 have been shown in p110α to bind ras and p85, respectively. The lipid kinase domain includes the region of AGE-1 that shares extensive homology with all lipid kinases. The approximate location of each point mutation is shown relative to the putative AGE-1 domains predicted by the Pileup program.

Figure 6 is the amino acid sequence of an AGE-1 polypeptide (SEQ ID NO: 1). The highlighted amino acid positions are preferably unchanged in AGE-1 variants (based on their identity with amino acids in the human kinase p110α).

Detailed Description

As described in more detail below, a pheromone-induced neurosecretory signaling system in *C. elegans* triggers developmental arrest and a dramatic increase in longevity at the dauer diapause stage. *age-1* is a key gene in this neuroendocrine pathway whose activity is required both for non-arrested development and for normal senescence. As shown herein, *age-1* encodes a member of the p110 family of phosphatidylinositol 3-kinase (PI 3-kinase) catalytic subunits. Four *age-1* mutant alleles affect this PI 3-kinase homologue: two lesions are stop codons that truncate the protein at distinct locations N-

terminal to the kinase domain and thus are likely to define the *age-1* null phenotype. Maternal *age-1* activity is specifically abrogated in one *age-1* mutant which was isolated on the basis of its enhanced longevity. Lack of either maternal or zygotic *age-1* gene activity confers long life-span but not developmental arrest whereas lack of both maternal and zygotic activity causes arrest at the dauer stage. These data suggest that decreased AGE-1-mediated phosphatidyl-inositol(3,4,5)P_3 (PIP_3) signaling leads to increased longevity, whereas complete lack of this signaling leads to developmental arrest.

Diapause in C. elegans and Other Organisms

In many animal phyla, neural signaling pathways couple sensory input to endocrine control of physiology and development. For example, many invertebrates arrest or alter their development in response to neuronal signals triggered by pheromones, light/dark cycles, or temperature (Tauber, M.J. et al., *Seasonal Adaptation of Insects* (New York, NY, 1986), Wilson, E.O., *The Insect Societies* (Cambridge, MA, 1972)). The nematode *Caenhorabditis elegans* arrests development at the dauer diapause stage after particular sensory neurons are exposed to a dauer-inducing pheromone (Riddle, D.L. in *The Dauer Larva in The Nematode Caenorhabditis elegans*. (ed. Wood, W.B.) 393-412 (Cold Spring Harbor, NY, 1988)). The formation of a dauer larva includes behavioral, physiological, and morphological changes: dauer larvae suspend the molting cycle and germ line development, stop feeding but initiate dispersal behavior, and secrete a specialized sealed cuticle (Riddle, D.L. in *The Dauer Larva in The Nematode Caenorhabditis elegans*. (ed. Wood, W.B.) 393-412 (Cold Spring Harbor, NY, 1988)). When pheromone levels decrease, the dauer recovers and re-enters a normal feeding and molting cycle to produce fertile adult animals. Dauer formation also interrupts normal senescence in *C. elegans*. Dauer arrested larvae can survive more than eight times the life-span of non-dauer animals without affecting life-span after recovery from the dauer stage (Riddle, D.L. in *The Dauer Larva in The Nematode Caenorhabditis elegans*. (ed. Wood, W.B.) 393-412 (Cold Spring Harbor, NY, 1988)). Because of the global

morphological and longevity changes associated with dauer formation, and by analogy with known endocrine control of diapause in other invertebrates (Williams, C.M., *Biol. Bull.* **103**:120-138 (1952)), it is likely that a neuroendocrine pathway is coupled to the sensory neurons that detect the dauer pheromone.

Many mutations affecting dauer formation (*daf* mutations) have been isolated and characterized (Riddle, D.L. et al., *Nature* **290**:668-671 (1981); Vowels, J.J. & Thomas, J.H., *Genetics* **130**:105-123 (1992); Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)). These fall into two main classes: dauer constitutive (*daf-c*) mutations, which cause animals to enter the dauer stage even in the absence of dauer-inducing pheromone, and dauer defective *(daf-d)* mutations, which prevent dauer formation even under conditions of high pheromone. The genes identified by these mutations have been ordered into a genetic epistasis pathway that is likely to represent the steps in the development or function of a neuroendocrine system composed of pheromone-sensing neurons, secretory cells, and target tissues (Riddle, D.L. et al., *Nature* **290**:668-671 (1981); Vowels, J.J. & Thomas, J.H., *Genetics* **130**:105-123 (1992); Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994); Thomas, J.H. et al., *Genetics* **134**:1105-1117 (1993)). Two of these genes, *daf-1* and *daf-4* encode homologues of TGF-β receptors, implicating this signaling pathway in pheromone signal transduction (Georgi, L.L. et al., *Cell* **61**:635-645 (1990); Estevez, M. et al., *Nature* **365**:644-649 (1993)).

Among the many genes that mediate the function of the dauer neuroendocrine pathway, *daf-2*, *daf-16*, and *daf-23* have been most directly implicated in regulation of longevity (Kenyon, C. et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995); Dorman, J.B. et al., *Genetics* **141**:1399 (1995)). While strong *daf-2* alleles and non-maternally rescued strong *daf-23* alleles induce dauer formation in the absence of pheromone, temperature sensitive *daf-2* alleles or maternally rescued *daf-23* alleles increase longevity two to three fold without forming dauer larvae (Kenyon, C.

et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995)). This suggests that the regulation of senescence can be decoupled from dauer formation. *daf-16* mutations suppress both the dauer constitutive phenotype and the increase in longevity of *daf-2* and *daf-23* mutants (Kenyon, C. et al., Nature **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995); Dorman, J.B. et al., *Genetics* **141**:1399 (1995)). Other *daf* mutations do not affect longevity, and are not efficiently suppressed by *daf-16* mutations (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994); Kenyon, C. et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995)). This genetic epistasis analysis suggests that the longevity-regulating *daf-2*, *daf-16*, *daf-23* subpathway acts either downstream or in parallel to the TGF-β signaling component of the dauer pathway (Vowels, J.J. & Thomas, J.H., *Genetics* **130**:105-123 (1992); Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)).

AGE-1 Cloning and Analysis

Age-1(hx546) was isolated in a genetic screen for increased longevity (Klass, M., *Mech Aging Dev* **22**:279-286 (1983); Friedman, D.B. & Johnson, T.E., *Genetics* **118**:75-86 (1988)). *Age-1(hx546)* animals live twice as long as wild type, and this increase in longevity is suppressed by mutations in *daf-16*, like the increased longevity phenotype of *daf-2* and *daf-23* mutants (Kenyon, C. et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995); Dorman, J.B. et al., *Genetics* **141**:1399 (1995)). Recently, Inoue and Thomas showed that at 27°C, a temperature above those routinely used in laboratory culture, *age-1(hx546)* has a dauer constitutive phenotype and fails to complement *daf-23* dauer constitutive alleles. Because strong *daf-23* alleles (e.g., *m333* and *mg44*) are haploinsufficient at 27°C (data not shown), complementation tests at that temperature are difficult to interpret. We complementation tested *age-1(hx546)* and dauer constitutive *daf-23* alleles at lower temperatures where there is no such haploinsufficiency (Figure 1). The results indicated that the *age-1(hx546)* fails to complement three *daf-23* dauer constitutive mutant alleles, and maps to

the same genetic interval. *Age-1* was therefore assigned both the increased longevity allele *(hx546)* and the dauer constitutive mutant alleles *(mg44, mg55, m333, mg109)* previously referred to as *daf-23* because the defining mutant allele was named *age-1* (Klass, M., *Mech Aging Dev* **22**:279-286 (1983); Friedman, D.B. & Johnson, T.E., *Genetics* **118**:75-86 (1988)). As shown below, *age-1(hx546)* is specifically defective in maternal *age-1* gene activity, whereas the stronger dauer constitutive *age-1* alleles are defective in both maternal and zygotic *age-1* gene activity.

The *age-1(mg44)* null allele (see below) marked in *cis* with *sqt-1(sc13)* was used in this genetic analysis (Figure 1). Animals bearing this *age-1* allele normally arrest development as dauer larvae only if they receive no maternal or zygotic *age-1* contribution (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)). For example, *age-1(mg44)* daughters of +/ *age-1(mg44)* mothers develop into fertile adults that then produce a brood of arrested *age-1(mg44)* dauer larvae (Figure 1). The *age-1(hx546)* mutation disrupts this maternal rescue: *age-1(mg44)* daughters of *age-1(hx546)/age-1(mg44)* mothers arrest development as dauers (Figure 1). A similar lack of *age-1(hx546)* maternal rescue was observed for *age-1(m333),* another probable null allele (see below) and *age-1(mg109)* (data not shown). Consistent with disruption of only maternal *age-1* expression by the *age-1(hx546)* mutation, a paternally contributed *age-1(hx546)* allele can zygotically rescue the dauer constitutive phenotype of progeny of *age-1(mg44)* homozygous mothers: *age-1(hx546)/age-1(mg44)* daughters of a mating of *age-1(hx546)* males to *age-1(mg44)/age-1(mg44)* hermaphrodites did not arrest development as dauer larvae, analogously to +/*age-1(mg44)* progeny from a mating to wild type males (Figure 1). Genetic mapping placed *age-1(hx546)* in the same 1.2 map unit genetic interval as *daf-23* (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)), between *sqt-1* and *lin-29* (Figure 1).

As shown by previous genetic analysis (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)) and by the molecular analysis below, the dauer constitutive

phenotype of most *age-1* alleles is the probable null phenotype. *age-1(hx546)* was not dauer constitutive at most temperatures (Klass, M., *Mech Aging Dev* **22**:279-286 (1983); Friedman, D.B. & Johnson, T.E., *Genetics* **118**:75-86 (1988)), probably because the zygotic *age-1* gene activity of this allele was sufficient to allow non-arrested development. This mutant may be long-lived because the decrease in maternally contributed *age-1* causes a decrease in the rate of senescence. Similarly, lack of zygotic expression in maternally rescued *age-1(m333)* homozygous progeny of +/*age-1(m333)* parents also leads to life-span extension without dauer arrest (Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995)). These animals had only maternal *age-1* activity, since *age-1(m333)* is a null mutant (see below). Thus, normal senescence is likely to depend on both maternal and zygotic *age-1* activity: in the absence of zygotic *age-1* gene activity or reduced maternal *age-1* gene activity, life-span increases. In the absence of both zygotic and maternal *age-1* gene activity, animals arrest at the dauer stage.

The data illustrated in Figure 1 is presented in tabular form below (Table 1).

TABLE 1 Regulation of lifespan and dauer formation by age-1

			Age phenotype		
Paternal genotype	Maternal genotype	Zygotic genotype	Dauer formation	Lifespan (days)	Lifespan relative to wild type
	+/+	+/+	non-dauer (1000)	8.1±0.2 (90)	1.0
	mg44/+	*mg44/mg44*	non-dauer (505)*	20.7±0.2 (26)	2.6
	mg44/mg44	*mg44/mg44*	non-dauer (505)		
	hx546/hx546	*hx546/hx546*	non-dauer (1000)	16.6±1.0 (43)	2.0
+/+	*mg44/mg44*	*mg44/+*	non-dauer (100)	10.7±0.5 (35)	1.3
hx546/hx546	*mg44/hx44*	*hx546/mg44*	non-dauer (100)	19.8±1.1 (54)	2.4
	mg44/hx546	*mg44/mg44*	dauer (406)		

The *age-1(mg44)* chromosome is marked in *cis* with *sqt-1(scl3)*, and *age-1(mg44)/+* strains have the *mnC1* balances chromosome as the + chromosome. Lifespan at 25°C is measured from the L4 stage to the last day that animals respond to light touch, and is presented as the mean ± s.e. Note that *sqt-1(sc13) age-1(mg44)* progeny of *age-1(hx546)/sqt-1(sc13) age-1(mg44)* form dauer larvae with 100% penetrance at 25°C. This is the same as control *age-1(mg44)* daughters *of age-1(mg44)* parents. The dauer larvae produced in both cases have the dark intestine, pharyngeal and cuticular remodelling, and arrest of the moulting cycle characteristic of this stage[3,5]. In contrast, 99% of *sqt-1(sc13) age-1(mg44)* progeny of *sqt-1(sc13) age1(mg44)/+* do not form dauer larvae, showing that maternal

age-1 activity is sufficient to allow non-dauer development. These animals live 2.6-fold longer than wild-type worms. Zygotic *age-1(+)* in the absence of maternal *age-1* can supply sufficient activity for normal lifespan and non-dauer development, but zygotic *age-1(hx546)* does not supply sufficient activity for normal longevity. Similar results were observed with other *age-1* alleles *m333* and *mg109* (data not shown). We three-factor mapped *age-1(hx546)* to the 1.2 map unit *sqt-1 lin-29* interval. Of 20 recombinants in this interval, 4 were between *sqt-1* and *age-1(hx546)*, and 16 were between *age-1* and *lin-29*. This shows that *age-1(hx546)* maps to the same genetic interval as *age-1(mg44)*. Consistent with this mapping, Sqt recombinants of the genotype *sqt-1(sc13) age-1(mg44)/sqt-1(sc13) age-1(hx546)* were isolated from *sqt-1(sc13) age-1(mg44)/age-1(hx546)* worms. Wild-type or *age-1 (hx546)* males were mated into *sqt-1(sc13) age-1 (mg44)* hermaphrodites. Cross progeny were picked to separate plates at 25°C and F_2 progeny were scored 3 days later. For genetic mapping, recombinants were picked from *age-1(hx546)/sqt-1(sc13) lin-29(n333)* hermaphrodites. Recombinants were tested for failure to maternally complement *age-1(m333)* for dauer formation at 25°C.

*1% of these worms form dauer larvae, and 20% become sterile adults.

The 1.2 map unit *sqt-1 lin-29* interval to which *age-1* maps consists of approximately 700 kb of DNA on 4 contigs (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)). The physical and genetic maps were correlated in this region by using five small chromosome II deficiencies, some of which complement and some of which fail to complement mutations in *age-1*. *Age-1* was determined to lie to the right of or on cosmid C24F2 because *mnDf75*, *mnDf76*, and *mnDf86* deficiencies, which fail to complement *sqt-1* but complement *age-1*, had breakpoints detectable by cosmid C24F2 (Figure 2A). *Age-1* was physically mapped to the left of the cloned marker *kin-6* (which lies on cosmid C46F8) by using PCR to determine that dead eggs homozygous for *mnDf90*, a deficiency which fails to complement both *sqt-1* and *age-1*, contained the DNA for *kin-6* whereas they deleted *sqt-1* (Figure 2A). These deficiencies mapped *age-1* to an approximately 240 kb interval between cosmid clones C24F2 and C46F8. Using cosmids from this interval as probes on Southern blots, we searched for a breakpoint associated with the *age-1(mg55)* gamma-ray-induced allele. Because *age-1(mg55)* showed pseudolinkage to chromosome I in genetic crosses (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)), this allele was suspected to be associated with a translocation and was the best *a priori* candidate allele to show a breakpoint in *age-1*. Probes from cosmid B0334 and probes from genomic clones overlapping the rightmost region in B0334 detected a novel breakpoint in *age-1(mg55)*/mnC1 but not in control DNAs carrying the same mnC1 balancer chromosome (Figures 2B and 2C).

The *C. elegans* Genome Project (Sulston, J. et al., *Nature* **356**:37-41 (1992)) has sequenced cosmid B0334. Analysis of the DNA sequence in the 4 kb region that detected the *age-1(mg55)* breakpoint revealed two putative exons that showed strong sequence identity with the last 88 amino acids of mammalian phosphatidylinositol 3-kinase (PI 3-kinase) p110 catalytic subunit (Hiles, I.D. et al., *Cell* **70**:419-429 (1992)). The region to the right of B0334 expected to contain the rest of *age-1* was not cloned in cosmids or phage by the *C. elegans* genome project. We isolated genomic phage and cDNA clones extending to the right from B0334 and used anchored polymerase chain reaction (PCR) of reverse transcribed RNA to isolate and determine the sequence of the coding region of *age-1* (Figure 2C). To confirm the splicing pattern of *age-1,* reverse transcription PCR (RTPCR) was used in conjunction with genomic sequencing of predicted splice junctions. The sequence predicted by cDNA clones and anchored PCR was further confirmed by sequencing genomic fragments corresponding to the predicted coding sequence. Because three independent cDNA clones end within 30 base pairs of each other and because these encode a protein coextensive with mammalian p110 (see below), we concluded that the assembled *age-1* cDNA was likely to be complete. The nucleic acid sequence of the *C. elegans age-1* cDNA is shown in Figure 4.

Analysis of the *age-1* DNA sequence revealed an open reading frame of 1185 amino acids. The *age-1* open reading frame bears four in-frame methionine residues that are potential translation start sites. While the second methionine shows a closer match to the *C. elegans* translation initiation consensus, the Kozak translation initiation rules favor the first methionine in an mRNA (Kozak, M., *Nucleic Acids Research* **15**:8125-8132 (1987); Kozak, M., *Proc. Natl. Acad. Sci.* **92**:2662-2666 (1995); Krause, M. in *Caenorhabditis elegans Modern Biological Analysis of an Organism* (ed. Epstein, H.F. & Shakes, D.C.) 483-512 (San Diego, CA, 1995)). Numbering from the first initiation codon, *age-1* was predicted to encode a 1146 amino acid protein (Figures 3 and 6). The *age-1* genomic region encoding this protein was sequenced from four *age-1* EMS-

induced alleles, *m333*, *mg44*, *mg109*, and *hx546*, revealing G->A point mutations (the predicted mutation from EMS mutagenesis) within this coding region in DNA isolated from three *age-1* alleles (see below). No change in the *age-1(hx546)* coding region or 3' UTR was detected. Because this mutation specifically affects maternal *age-1* activity, it may be located in a flanking transcriptional regulatory region not yet sequenced. Thus four *age-1* mutations, *mg55*, *m333*, *mg44*, and *mg109* affect this open reading frame, endorsing its assignment to *age-1* gene activity.

The AGE-1 protein is closely related to a family of mammalian phosphatidylinositol 3-kinase (PI 3-kinase) p110 catalytic subunits (Hiles, I.D. et al., *Cell* **70**:419-429 (1992); Kapeller R. & Cantley, L.C., *Bioessays* **16**:565-576 (1994)). PI 3-kinases generate a membrane-localized signaling molecule, phosphatidylinositol P_3 (PIP_3) (Riddle, D.L. in *The Dauer Larva in The Nematode Caenorhabditis elegans*. (ed. Wood, W.B.) 393-412 (Cold Spring Harbor, NY, 1988); Williams, C.M., *Biol. Bull.* **103**:120-138 (1952); Riddle, D.L. et al., *Nature* **290**:668-671 (1981)) that is thought to transduce signals from upstream receptors to as yet unknown effector molecules (Kapeller R. & Cantley, L.C., *Bioessays* **16**:565-576 (1994)). There are three known PI 3-kinase types. The α and β p110 types are targeted to activated receptor kinases by the regulatory p85 or p55 subunits (Kapeller R. & Cantley, L.C., *Bioessays* **16**:565-576 (1994)). These regulatory subunits have SH2 domains that recognize phosphorylated tyrosines on those receptors and other proteins (Kapeller R. & Cantley, L.C., *Bioessays* **16**:565-576 (1994); Liscovitch, M. & Cantley, L.C., *Cell* **81**:659-662 (1995); Carpenter, C.L. et al., *Molecular and Cellular Biology* **13**:1657-65 (1993); Dhand, R. et al., *EMBO Journal* **13**:511-21 (1994); Pons, S. et al., *Molecular and Cellular Biology* **15**:4453-65 (1995)). The p110α or p110β types bind to p85 via their N-terminal 130 amino acids (Kapeller R. & Cantley, L.C., *Bioessays* **16**:565-576 (1994); Liscovitch, M. & Cantley, L.C., *Cell* **81**:659-662 (1995); Carpenter, C.L., et al., *Molecular and Cellular Biology* **13**:1657-65 (1993)). The p110α can phosphorylate p85 subunits as well as lipids (Carpenter, C.L. et

al., *Molecular and Cellular Biology* **13**:1657-65 (1993); Dhand, R. et al., *EMBO Journal* **13**:511-21 (1994)); thus PI 3-kinases may also transduce signals via a protein kinase cascade. A third type of p110 subunit, p110γ, binds to heterotrimeric G-proteins, and presumably couples indirectly to serpentine receptors (Stoyanov, B. et al., *Science* **269**:690-3 (1995)).

Gap and Blast analysis (Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) indicated that AGE-1 is 29.6% identical to mouse p110α, 29.8% identical to human p110β, and 28.0% identical to human p110γ. Large segments of AGE-1 show up to 42% sequence identity to the p110 PI-3 kinase proteins in the kinase and upstream domains conserved in lipid kinases (Figures 3 and 5). Comparison of AGE-1 to the p110 proteins in the N-terminal 130 amino acids that mediate p110 α and β interaction with p85 (Dhand, R. et al., *EMBO Journal* **13**:511-21 (1994)) showed that AGE-1 is more related to p110α (22.0% identity) than to p110β (17.1% identity) or p110γ (9.8% identity) in this region. Many of the amino acid residues in this region that are conserved between AGE-1 and p110α, are also conserved between p110α and p110β, which share 45% amino acid identity in the region (Figure 3). The random probability of alignment of AGE-1 with these p110 kinases is extremely low: e^{-113} for p110α, e^{-101} for p110β, and e^{-93} for p110γ (versus, for example, a probability of random alignment with PI 4-kinase of e^{-22} or to DNA repair kinases of e^{-8}). The regions conserved in AGE-1 suggest that this protein may couple to a p85-like tyrosine kinase adaptor rather than to a $G_\beta\gamma$ like adaptor protein. However, over the entire protein, mouse p110α is more similar to the other mammalian p110 classes than to AGE-1 (mouse p110α is 42.0% identical to human p110β and 34.5% identical to human p110γ, vs. 30.1% identical to AGE-1), suggesting that AGE-1 may be a divergent PI 3-kinase class.

The *age-1* maternal effect dauer constitutive mutations are probable null alleles. *age-1(mg44)* is a Trp405Amber mutation that truncates AGE-1 upstream of the

kinase domain and most of the conserved regions (Figure 3). This mutation is a good candidate to define the *age-1* null phenotype. The *age-1(m333)* mutation is Trp659Opal that also truncates the AGE-1 protein upstream of most conserved domains, and is also a likely null allele. The *age-1(mg55)* breakpoint removes the C terminal portion of the kinase domain from the AGE-1 protein and *age-1* 3' UTR (Figure 2A). *Age-1(mg109)* causes an Ala845Thr substitution in a region of the protein that is conserved among AGE-1 and mammalian PI 3-kinases (Figure 3). Both AGE-1 and mammalian p110α have an Ala at this position, whereas p110β and γ have Lys at this position (Figure 3). The high degree of conservation and the severity of the *age-1(mg109)* phenotype suggests that this region performs an essential function in AGE-1.

All of these *age-1* alleles, including those that are predicted to truncate the AGE-1 protein, show a dauer constitutive phenotype which can be rescued by wild type maternal gene activity. In addition, the *age-1(m333)* allele, which is a probable null allele, shows a dramatic longevity increase when maternal but not zygotic *age-1* gene activity is supplied (Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995)). These data show that the AGE-1 PI 3-kinase homologue functions in the particular signaling pathway that controls dauer developmental arrest and senescence, and are not consistent with a more general AGE-1 requirement. In addition, this shows that maternal AGE-1-mediated phosphatidylinositol signaling is sufficient to rescue lack of zygotic AGE-1 signaling for arrest at the dauer stage but not for decreased senescence. Because in *age-1(hx546)*, reduced maternal AGE-1 phosphatidylinositol signaling also leads to increased longevity, these data suggest that normal senescence depends on phosphatidylinositol signaling from both maternal and zygotic AGE-1.

The strong sequence similarity that AGE-1 shares with mammalian PI 3-kinases suggests a variety of possible roles that PIP_3 signaling could play in regulating dauer development and senescence. In mammals, PI 3-kinase signaling has been implicated in neural development as well as in hormonal signaling. Nerve growth factor

signaling from the Trk kinase receptor and neurite outgrowth in PC12 cells are inhibited by the PI 3-kinase inhibitor wortmannin (Kimura, K. et al., *J. Biol. Chem* **269**:18961-7 (1994); Yao, R. & Cooper, G.M., *Science* **267**:2003-6 (1995)). PI 3-kinase has also been implicated in histamine secretion by mast cells (Yano, H. et al., *J. Biol. Chem.* **268**:25846-25856 (1993)) and in signal transduction downstream of the metabolic control hormone insulin (Levy-Toledano, R. et al., *J. Biol. Chem.* **269**:31178-31182 (1994)). Thus, there are precedents for PI 3-kinase function at many of the steps that are likely to be required in a neuroendocrine pathway, including development and differentiation of neurons and target tissues, secretory events, and signal transduction in target tissues.

AGE-1-mediated PIP_3 signaling could function in the development of the dauer neurosecretory pathway or in the transduction of pheromone signal in the pathway. Genetic epistasis analysis suggests that *age-1* may function downstream of the *daf* genes involved in sensory processing of the dauer pheromone signal, for example in the development or function of secretory neurons or target tissues (Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994)). The observation that *age-1* null mutants are maternally rescued for dauer arrest is consistent with an early *age-1* role. The dauer pheromone is normally detected during the L1 stage (Riddle, D.L. in *The Dauer Larva in The Nematode Caenorhabditis elegans*. (ed. Wood, W.B.) 393-412 (Cold Spring Harbor, NY, 1988)), suggesting that if the maternally supplied AGE-1 functions in pheromone signal transduction, the *age-1* mRNA, protein, or phosphatidylinositol signal itself must perdure from the germ line until this stage. If *age-1* mediates development of this pathway, it would be expected to function during embryogenesis when the neurons and target tissues likely to function in the dauer neuroendocrine pathway are generated.

The longevity phenotype of *age-1* mutants suggests a direct function of phosphatidylinositol signaling in senescence. Disruption of *age-1* gene activity confers long life span in the absence of dauer entry, suggesting that the longevity of these mutants is not a simple consequence of dauer entry. More likely, reduced PI 3-kinase

activity triggers a subset of the dauer program that includes a decrease in the rate of senescence but not developmental arrest. Free radical byproducts of aerobic metabolism have been suggested to contribute to senescence by directly damaging a variety of essential molecules, including DNA, proteins, and lipids (Finch, C.E., *Longevity, Senescence, and the Genome* (Chicago, IL 1990)). If phosphatidylinositol signaling from AGE-1 normally regulates the level of free radicals, then decreases in *age-1* gene activity in mutants or dauer larvae could lead to increased longevity. In fact, *age-1* mutants and dauer larvae show increased levels of catalase and superoxide dismutase and resistance to free radical generating drugs and treatments (Larsen, P., *Proc. Natl. Acad. Sci.* **90**:8905-8909 (1993); VanFleteren, J.R., *Biochem. J.* **292**:605-608 (1993)). Interestingly, a wortmannin-sensitive PI-3 kinase is necessary in the production of superoxide radicals in bacterial killing by mammalian neutrophils (Okada, T. et al., *J. Biol. Chem.* **269**:3563-3567 (1994); Thelen, M. et al., *Proc. Natl. Acad. Sci.* **91**:4960-4 (1994)).

The identification of *age-1* as a PI 3-kinase suggests that other genes in the dauer genetic pathway could identify the *in vivo* downstream targets of PIP_3 signaling. *daf-2* functions at the same point as *age-1* in the dauer and senescence epistasis pathway (Vowels, J.J. & Thomas, J.H., *Genetics* **130**:105-123 (1992); Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994); Kenyon, C. et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995); Dorman, J.B. et al., *Genetics* **141**:1399 (1995)). DAF-2 could be a downstream positively-regulated target of PIP_3 signaling, a PI 3-kinase regulatory subunit such as p85 or p55, or an upstream receptor. Candidates for positively-regulated downstream targets of PIP_3 signaling have been detected biochemically (Liscovitch & L.C. Cantley, L., *Cell* **77**:324-34 (1994); Toker, A. et al., *The Journal of Biological Chemistry* **269**:32358-67 (1994); Akimoto, K. et al., *The EMBO Journal* **15**:788-798 (1996); Jones, P.F. et al., *Proc. Natl. Acad. Sci.* **80**:4171-5 (1991); Franke, T.F. et al., *Cell* **81**:727-736 (1995); Burgering, B.M.T. & Coffer, P.J., *Nature* **376**:599-602 (1995)); the *in vivo* function of such candidates would be validated

by detection of *daf* gene homologues. No negatively regulated targets of PIP_3 signaling have been detected biochemically. Genetic data indicates that *daf-16* may be a negatively regulated target of PIP_3 signaling; the increase in longevity and arrest at the dauer stage in the *age-1* mutant that we argue here results from a loss in PIP_3 signaling is suppressed by mutations in *daf-16* (Vowels, J.J. & Thomas, J.H., *Genetics* **130**:105-123 (1992); Gottlieb, S. & Ruvkun, G., *Genetics* **137**:107-120 (1994); Kenyon, C. et al., *Nature* **366**:461-464 (1993); Larsen, P.L. et al., *Genetics* **139**:1567-1583 (1995); Dorman, J.B. et al., *Genetics* **141**:1399 (1995)). These observations suggest that under conditions of non-arrested normally senescing growth, the small membrane-bound PIP_3 product of AGE-1 negatively regulates DAF-16 activity to control life span in *C. elegans*.

Cloning Mammalian AGE-1 Polypeptides

Based on our isolation of a novel AGE-1 gene and cDNA, the isolation of additional mammalian AGE-1 nucleic acid sequences, including human AGE-1, is made possible using the sequence described herein and standard techniques. In particular, using all or a portion of the AGE-1 sequence, one may readily design AGE-1 oligonucleotide probes, including degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either strand of the DNA. Exemplary probes or primers for isolating mammalian AGE-1 sequences preferably correspond to conserved blocks of amino acids, for example, amino acids 852-864 (IFKNGDDLRQDML) (SEQ ID NO: 13) or amino acids 1111-1116 (HIDFGH) (SEQ ID NO: 14) of Fig. 6 (SEQ ID NO: 1). General methods for designing and preparing such probes are provided, for example, in Ausubel et al., *Current Protocols in Molecular Biology*, 1996, Wiley & Sons, New York, NY; and *Guide to Molecular Cloning Techniques*, 1987, S. L. Berger and A. R. Kimmel, eds., Academic Press, New York. These oligonucleotides are useful for AGE-1 gene isolation, either through their use as probes for hybridizing to AGE-1 complementary sequences or as primers for various polymerase chain reaction (PCR) cloning strategies.

If a PCR approach is utilized, the primers are optionally designed to allow cloning of the amplified product into a suitable vector.

Hybridization techniques and procedures are well known to those skilled in the art and are described, for example, in Ausubel et al., supra, and *Guide to Molecular Cloning Techniques*, supra. If desired, a combination of different oligonucleotide probes may be used for the screening of the recombinant DNA library. The oligonucleotides are, for example, labelled with ^{32}P using methods known in the art, and the detectably-labelled oligonucleotides are used to probe filter replicas from a recombinant DNA library. Recombinant DNA libraries (for example, human cDNA libraries) may be prepared according to methods well known in the art, for example, as described in Ausubel et al., supra, or may be obtained from commercial sources.

For detection or isolation of closely related AGE-1 sequences, high stringency hybridization conditions may be employed; such conditions include hybridization at about 42°C and about 50% formamide; a first wash at about 65°C, about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% SDS, 1X SSC. Lower stringency conditions for detecting AGE-1 genes having less sequence identity to the AGE-1 gene described herein include, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.

As discussed above, AGE-1 oligonucleotides may also be used as primers in PCR cloning strategies. Such PCR methods are well known in the art and described, for example, in *PCR Technology*, H.A. Erlich, ed., Stockton Press, London, 1989; *PCR Protocols: A Guide to Methods and Applications*, M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds., Academic Press, Inc., New York, 1990; and Ausubel et al., supra. Again, sequences corresponding to conserved regions in the AGE-1 sequence (for example, those regions described above) are preferred for use in isolating mammalian AGE-1 sequences.

AGE-1 Polypeptide Expression

In general, AGE-1 polypeptides according to the invention may be produced by transformation of a suitable host cell with all or part of an AGE-1-encoding cDNA fragment (e.g., one of the cDNAs described above) in a suitable expression vehicle.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The AGE-1 polypeptide may be produced in a prokaryotic host (e.g., E. coli) or in a eukaryotic host (e.g., Saccharomyces cerevisiae, insect cells, e.g., Sf9 or Sf21 cells, or mammalian cells, e.g., COS 1, NIH 3T3, or HeLa cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., supra). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

One preferred expression system is the baculovirus system (using, for example, Sf9 cells and the method of Ausubel et al., supra). Another baculovirus system makes use of the vector pBacPAK9 and is available from Clontech (Palo Alto, CA).

Alternatively, an AGE-1 polypeptide is produced in a mammalian system, for example by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the AGE-1 protein is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the AGE-1 protein-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection may be

accomplished in most cell types. Recombinant protein expression may be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR$^-$ cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

In yet other alternative approaches, the AGE-1 polypeptide is produced in vivo or, preferably, in vitro using a T7 system (see, for example, Ausubel et al., supra, or other standard techniques).

Once the recombinant AGE-1 protein is expressed, it is isolated, e.g., using affinity chromatography. In one example, an anti-AGE-1 protein antibody (e.g., produced as described herein) may be attached to a column and used to isolate the AGE-1 protein. Lysis and fractionation of AGE-1 protein-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra).

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, eds., Work and Burdon, Elsevier, 1980).

Polypeptides of the invention, particularly short AGE-1 polypeptide fragments, may also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL).

These general techniques of polypeptide expression and purification may also be used to produce and isolate useful AGE-1 fragments or analogs (described herein).

Anti-AGE-1 Antibodies

Using the AGE-1 polypeptide described above, anti-AGE-1 antibodies have been produced as follows. An AGE-1 cDNA fragment encoding amino acids 1089-1164 was fused to GST, and the fusion protein produced in E. coli by standard techniques. The fusion protein was then purified on a glutathione column, also by standard techniques, and was used to immunize rabbits. The antisera obtained was then itself purified on a GST-AGE-1 affinity column by the method of Finney and Ruvkun (*Cell* **63**:895-905, 1990). This antisera was shown to specifically identify GST-AGE-1 by Western blotting.

Other AGE-1-specific antibodies may be produced by this or alternative techniques. For example, the AGE-1 polypeptides described herein (or immunogenic fragments or analogs) may be used to raise other polyclonal antisera or monoclonal antibodies; one particular alternative immunogenic fragment is represented by AGE-1 amino acids 550 to 965. Polypeptides for antibody production may be produced by recombinant or peptide synthetic techniques (see, e.g., *Solid Phase Peptide Synthesis*, supra; Ausubel et al., supra).

For polyclonal antisera, the peptides may, if desired, be coupled to a carrier protein, such as KLH as described in Ausubel et al, supra. The KLH-peptide is mixed with Freund's adjuvant and injected into guinea pigs, rats, or preferably rabbits. Antibodies may be purified by any method of peptide antigen affinity chromatography.

Alternatively, monoclonal antibodies may be prepared using an AGE-1 polypeptide (or immunogenic fragment or analog) and standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., *In Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, NY, 1981; Ausubel et al., supra).

Once produced, polyclonal or monoclonal antibodies are tested for specific AGE-1 recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize AGE-1 are

considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay to measure or monitor the level of AGE-1 produced by a mammal or to screen for compounds which modulate AGE-1 production. Anti-AGE-1 antibodies may also be used to identify cells that express the AGE-1 gene.

Samples obtained from the AGE-1 nonsense alleles, m333 and mg44 (described above), provide useful negative controls for antisera specificity.

Identification and Administration of Molecules that Modulate AGE-1 Expression or Activity

Isolation of an AGE-1 cDNA and knowledge of its involvement in the aging process also facilitates the identification of molecules which decrease AGE-1 expression or activity (i.e., AGE-1 antagonists). According to one approach, AGE-1 expression is measured following the addition of antagonist molecules to the culture medium of AGE-1-expressing cells. Alternatively, the candidate antagonists may be directly administered to animals (for example, nematodes or mice) and used to screen for antagonists.

AGE-1 expression is then measured, for example, by standard Northern blot analysis (Ausubel et al., supra) using an AGE-1 nucleic acid (or fragment) as a hybridization probe. The level of AGE-1 expression in the presence of the candidate molecule is compared to the level measured for the same cells in the same culture medium or test animal, but in the absence of the candidate molecule. Preferred modulators for anti-aging purposes are those which cause a decrease in AGE-1 expression.

Alternatively, the effect of candidate modulators on expression may be measured at the level of AGE-1 protein production using the same general approach in combination with standard immunological detection techniques, such as Western blotting or immunoprecipitation with an AGE-1-specific antibody (for example, the AGE-1 antibody described herein). Again, useful anti-aging modulators are identified as those which produce a decrease in AGE-1 polypeptide production.

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (e.g., an extract or supernatant obtained from cells). In a mixed compound assay, AGE-1 expression is tested against progressively smaller subsets of the candidate compound pool (e.g., produced by standard purification techniques, e.g., HPLC or FPLC; Ausubel et al., supra) until a single compound or minimal compound mixture is demonstrated to modulate AGE-1 expression.

Alternatively, or in addition, candidate compounds may be screened for those which antagonize native or recombinant AGE-1 activity. In a preferred approach, kinase activity (for example, PI 3-kinase activity) in the presence of, or after treatment with, a candidate compound is compared to activity in its absence under equivalent conditions. Again, such a screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion.

Kinase activity may be measured by any standard assay, for example, it may be measured by monitoring the ability of the enzyme to transfer ^{32}P-ATP to a PIP substrate on a TLC plate (as described, for example, by Whitman et al., *Nature* **322**:644-646, 1988), or it may be measured by the method of Kimura et al. (*J. Biol. Chem.* **269**:18961-18967, 1994). If desired, prior to assaying activity, the enzyme may be isolated from a sample, for example, by immunoprecipitation with an AGE-1-specific antibody. The AGE-1 mutants described herein (for example, mg44, m333, and mg109) have reduced activity in these in vitro assays and may be used as control samples.

Candidate AGE-1 antagonists include peptide as well as non-peptide molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured). Because the most likely AGE-1 substrate is PIP_2 and its product PIP_3, drugs that mimic the transition state between PIP_2 and PIP_3 (so called, transition analogs) are good candidates for compounds which down-regulate the AGE-1-mediated synthesis of PIP_3,

and thereby increase longevity. Because both the AGE-1 substrate and product are membrane bound molecules, drugs that interfere with AGE-1 action may be hydrophobic, decreasing or eliminating problems commonly associated with membrane permeability.

Antagonists found to be effective at the level of cellular AGE-1 expression or activity may be confirmed as useful in animal models (for example, nematodes or mice).

A molecule which promotes a decrease in AGE-1 expression or AGE-1 activity is considered particularly useful in the invention; such a molecule may be used, for example, as a therapeutic to decrease the level or activity of native, cellular AGE-1 and thereby increase the longevity of the host animal (for example, human).

An AGE-1 antagonist for therapeutic use may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer AGE-1 to patients. Although intravenous administration is preferred, any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for AGE-1

antagonists include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with an AGE-1 antagonist may be combined with any other anti-aging therapy.

AGE-1 Pesticides

AGE-1 antagonists may also be used as novel pesticides, for example, to control insects or nematodes. Because AGE-1 controls diapause, compounds which antagonize its action may be used to trigger diapause inappropiately, with a concomitant suspension of feeding behavior and reproduction (Tauber et al., Seasonal Adaptation of Insects, 1986, New York, NY, Oxford University Press, p. 411). Such pesticides, which target invertebrate diapause events, are useful for enhancing agricultural production with fewer human health hazards than current neurotransmitter-based pesticides.

Determination of Longevity

Due to their role in aging, AGE-1 polypeptides and nucleic acid sequences are useful for determining the longevity of an organism (for example, a human). In particular, because decreased AGE-1 correlates with increased longevity, a decrease in the level of AGE-1 production or activity provides an indication that a host organism will age more slowly or have an increased life-span. Levels of AGE-1 expression or its activity may be assayed by any standard technique.

For example, expression in a biological sample may be monitored by standard Northern blot analysis or may be aided by PCR (see, e.g., Ausubel et al., supra; PCR Technology: Principles and Applications for DNA Amplification, ed., H.A. Ehrlich, Stockton Press, NY).

Alternatively, immunoassays may be used to detect or monitor the level of AGE-1 protein. AGE-1-specific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to measure AGE-1 polypeptide levels; again comparison is to wild-type AGE-1 levels, and a decrease in AGE-1 production is indicative of increased longevity. Examples of immunoassays are described, e.g., in Ausubel et al., supra. Immunohistochemical techniques may also be utilized for AGE-1 detection. For example, a tissue sample may be obtained from an individual, and a section stained for the presence of AGE-1 using an anti-AGE-1 antibody and any standard detection system (e.g., one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in, e.g., Bancroft and Stevens (Theory and Practice of Histological Techniques, Churchill Livingstone, 1982) and Ausubel et al. (supra).

Mismatch detection assays also provide the opportunity to detect AGE-1 mutations and thereby determine longevity. This type of approach may also be used to detect AGE-1 variants in prenatal screens.

Finally, a combined method may be employed that begins with an evaluation of AGE-1 protein production (for example, by immunological techniques) and also includes a nucleic acid-based detection technique designed to identify more subtle AGE-1 mutations (for example, point mutations). A number of standard mismatch detection assays are available to those skilled in the art, and any preferred technique may be used. By this approach, mutations in AGE-1 may be detected that either result in loss of AGE-1 expression or loss of AGE-1 biological activity. In a variation of this combined method, AGE-1 activity (rather than production) is measured as kinase activity using any appropriate kinase assay system (for example, the assays of Whitman et al., Nature 322:644-646 (1988); or Kimura et al., J. Biol. Chem. 269:18961-18967 (1994)).

AGE-1 Interacting Polypeptides

The isolation of AGE-1 sequences also facilitates the identification of polypeptides which interact with the AGE-1 protein. Such polypeptide-encoding sequences are isolated by any standard two hybrid system (see, for example, Fields et al., *Nature* **340**:245-246 (1989); Yang et al., *Science* **257**:680-682 (1992); Zervos et al., *Cell* **72**:223-232 (1993)). For example, all or a part of the AGE-1 sequence may be fused to a DNA binding domain (such as the GAL4 or LexA DNA binding domain). After establishing that this fusion protein does not itself activate expression of a reporter gene (for example, a lacZ or LEU2 reporter gene) bearing appropriate DNA binding sites, this fusion protein is used as an interaction target. Candidate interacting proteins fused to an activation domain (for example, an acidic activation domain) are then co-expressed with the AGE-1 fusion in host cells, and interacting proteins are identified by their ability to contact the AGE-1 sequence and stimulate reporter gene expression. False positive interactions are eliminated by carrying out a control experiment with an unrelated tester protein fused to an equivalent activation domain (or, if desired, a large panel of such tester proteins).

Once AGE-1 protein-protein interactions are identified, compounds may be screened for those which disrupt the interaction. These compounds provide good candidates for AGE-1 antagonists and may be tested or confirmed by any of the approaches described above. Compounds identified by this method may be used for any of the above-described purposes.

Other Embodiments

In other embodiments, the invention includes any protein which is substantially identical to the AGE-1 polypeptide of Fig. 6 (SEQ ID NO: 1); such homologs include other substantially pure naturally-occurring mammalian AGE-1 polypeptides (for example, the human AGE-1 polypeptide) as well as allelic variants;

natural mutants; induced mutants; proteins encoded by DNA that hybridizes to the AGE-1 DNA sequence of Fig. 4 (SEQ ID NO: 2) under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2X SSC at 40°C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to an AGE-1 polypeptide.

The invention further includes analogs of any naturally-occurring AGE-1 polypeptide. Analogs can differ from the naturally-occurring AGE-1 protein by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 50%, more preferably 60%, and most preferably 85% or even 95% identity with a naturally-occurring AGE-1 amino acid sequence. Modifications include in vivo and in vitro chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring AGE-1 polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the invention also includes AGE-1 polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of AGE-1 polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of

amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events). Preferable fragments according to the invention include, without limitation, amino acids amino acids 387-641, 387-1146, 1-130, 1-150, 1-658, or 1-404 of Figure 6 (SEQ ID NO: 1).

For certain purposes, all or a portion of the AGE-1 polypeptide sequence may be fused to another protein (for example, by recombinant means). In one example, AGE-1 may be fused to the green fluorescent protein, GFP (Chalfie et al., *Science* **263**:802-805, 1994). Such a fusion protein is useful, for example, for monitoring the expression level of AGE-1 in vivo (for example, by fluorescence microscopy) following treatment with candidate or known AGE-1 antagonists.

The methods of the invention may be used to determine longevity or screen for AGE-1 modulatory compounds in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the AGE-1 polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

Other embodiments are within the following claims.

What is claimed is: